

## Designing biopolymer fluid gels : a microstructural approach

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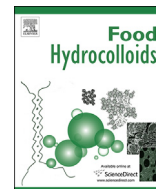
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## Designing biopolymer fluid gels: A microstructural approach

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## ABSTRACT

Fluid gels are suspensions of gelled particles whose elasticity and physical dimensions can be manipulated to impart a range of material properties. The formation and physical properties of fluid gels are discussed with the focus being on the process–structure relationship.

Recent work in this area has led to the development of new techniques for the production of thermo-stable fluid gels. An overview is provided on these techniques which have been successfully employed to produce thermo-stable fluid gels from both alginate and proteins.

An enhanced understanding of the molecular events occurring during fluid gel formation is outlined which has enabled greater control over the microstructure. This has allowed the production of small ( $<10\ \mu\text{m}$ ), spherical and reasonably soft particles which resemble oil droplets. These particles when used in emulsion based products to replace a significant proportion of the oil, show rheology matching that of the full fat original product.

Increasing the volume fraction and elasticity of fluid gel particles has been shown to decrease the friction measured at the contact between the soft surfaces of a tribometer. This ability of fluid gel particles to provide lubrication through entrainment to the contact zone suggests that fluid gels have the potential to impart sensory attributes typically associated with fat.

Directions for future research are also discussed which may provide further insights into food structuring and breakdown thereby assisting the design of more complex structures with novel functional attributes.

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## 1. Introduction

Biopolymers have traditionally been used in the food industry to provide structural stability and to impart desirable texture to a wide range of products including emulsions. The relatively recent rise in health problems associated with obesity and consumer demand for tasty products has led scientist to develop low-energy food products that are acceptable in terms of sensory attributes.

Microstructural engineering has emerged as a powerful tool to meet these demands, where polysaccharides are often used to build structures that resemble those of the original product that is to be reformulated. To achieve this, methods have been developed to enable scientists to control and manipulate the physical properties of structuring ingredients. An example of this is the production of suspensions of micro-gelled particles whose strength and physical dimensions are tailored to match the structures of the ingredients

they are replacing such as oil droplets. The colloidal properties of those particulate suspensions (i.e. fluid gels) allow them to impart desirable properties in emulsion based products.

The production of fluid gels was restricted to polysaccharides, specifically polysaccharides which undergo thermo-reversible ordering transitions, but recent developments have enabled the production of thermally stable fluid gels. This have allowed for a much wider range of applications in the food industry. Thermo-stable fluid gels have been created by calcium-induced gelation of alginates and also with whey protein materials. Methods for the production of those fluid gels will be described in this paper with the focus being on the relationship between processing conditions and the resulting microstructures.

Key requirements for successful design of colloidal structures for fat replacement are advance the understanding of the mechanisms which underlie the functional properties of oil droplets and the ability to predict their behaviour using instrumental techniques. Oil droplets in food products are responsible for desirable sensory attributes such as creaminess and to impart flavour. Textural attributes are strongly dependent upon the level of lubrication provided

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by the material in the contact generated between the tongue and palate during consumption. For that reason and in order to characterise the lubricating behaviour of fluid gels, soft-tribological techniques have been used, where the friction between the soft surfaces of a ball and disc in relative motion is measured. Tribological results from oil-in-water emulsions were shown to correlate well with sensory data, providing an insight into the processes occurring in the oral cavity during food consumption. This correlation relates to the fact that the structure of the emulsion deforms into a thin film during the last step of food processing in the mouth, specifically as a result of the movement of the tongue against the palate before swallowing. The dimensions of the thin film that is generated during oral processing is similar to that which forms between the ball and the disc of the tribometer at speeds between 10 mm/s and 100 mm/s (Malone, Appelqvist, & Norton, 2003). Thus, a good match has been found between sensory measurements and the tribological data obtained at these speeds, when soft rubber materials that resemble the surfaces of the oral cavity are used.

Fluid gels have shown the ability to generate thin film in these flow conditions. As such, fluid gel tribology is specifically relevant to determination and prediction in mouth sensory attributes by studying the ability of fluid gels to provide lubrication.

Recent work on fluid gel lubrication will also be covered, with a focus on aspects that are of importance for microstructural engineering including the influence of particle structure on particle entrainment into the contact zone and the resulting level of lubrication.

A discussion on the areas of future research that are required to gain a better understanding of fluid gel structuring and breakdown in both the mouth and the gastrointestinal (GI) tract is provided. This will enable the design of novel and more complex fluid gel structures. A multidisciplinary approach including knowledge of colloid science, sensory response and physiology will be needed to the exciting challenge of bringing an insight into how foods react to biological processes, how they affect organoleptic perception and their impact on human health.

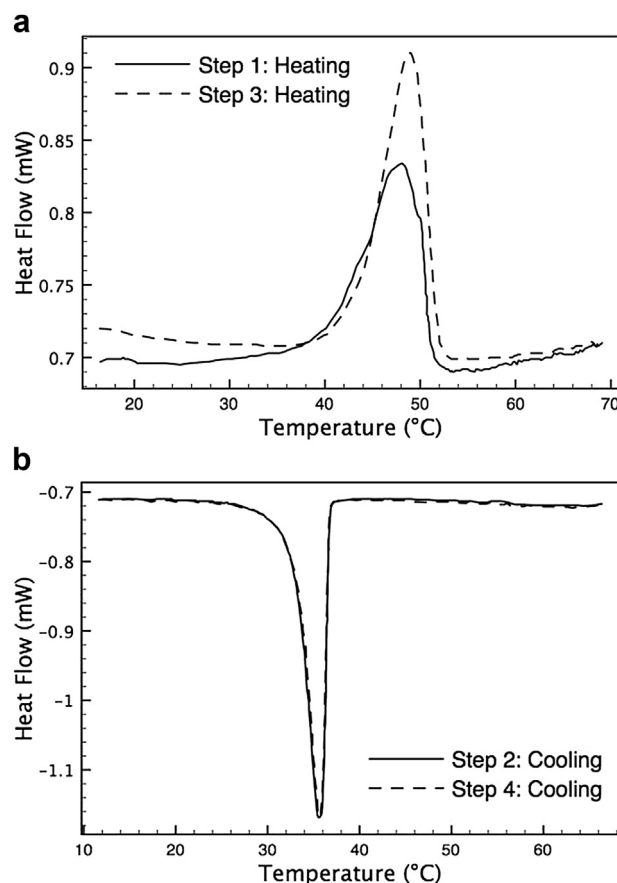
## 2. Thermo-reversible fluid gels from polysaccharides

Fluid gels are formed by the application of a flow field to the gelling biopolymer solution while undergoing conformational transition and subsequent aggregation. For biopolymers undergoing thermally reversible transitions, control over the kinetics of the ordering process is achieved by the manipulation of the cooling rate which determines the final particle physical properties. Kinetic competition between the processes of network formation and shear-induced break-up that occurs as the midpoint temperature is approached has a dramatic effect on the resulting particle structure (size, shape) and the intrinsic particle 'reactivity'. At high cooling rates, the gelation process dominates over the applied shear rate and large irregularly-shaped particles are produced. As the conformational ordering kinetics is more closely matched to the time scale of the applied shear rate (i.e. at lower cooling rates) the input from the applied flow field increases, resulting in the formation of small and spherical particles. Consequently, systems exhibit a more fluid-like response (reduced storage modulus) than that of fluid gels produced at higher cooling rates. Under the same applied cooling rate, both particle size and volume occupancy are strongly influenced by the applied shear field: increasing the magnitude of the shear rate leads to smaller particles and lower volume fractions. Work by Gabriele, Spyropoulos, and Norton (2009) showed that while big and irregularly-shaped particles are produced at low shear rates, small and nearly spherical particles can be obtained at high shear rates for  $\kappa$ -carrageenan fluid gels produced at the same cooling rates (Gabriele et al., 2009).

Therefore, a range of microstructures with significantly different physical properties can be produced from minor changes to the processing parameters whilst using the same material.

Mechanistically, the formation of fluid gels is a nucleation and growth process (Norton, Jarvis, & Foster, 1999). The applied shear forces ensure the initially formed gel nuclei remain separated from each other which limits the molecular ordering to occur within distinct particles.

The resulting particles have a higher polymer density at centre which gradually decreases towards the edge due to the weak interfacial tension acting between the gelled and non-gelled phases of the polymer and also that the input from the flow field is higher at the particle surface. However, as will be discussed later in this paper, the degree to which the applied shear limits molecular ordering depends largely on the nature of the polymer. Both the ordering rate and the cohesive nature of the bonds (e.g. weak hydrogen bonds or ionic crosslinking) determine the gelled polymeric network of the individual particles and consequently their stiffness. The polymeric network of thermo-reversible fluid gel particles can be elucidated by comparing the melting profile of a fluid gel with that of the corresponding quiescently cooled gel. The experimental procedure consists of measuring the thermal transitions when the fluid gel is heated above its melting temperature, then cooled and re-heated again. As such, the disordering transition that occurs during the first heating cycle (melting the fluid gel) can be assessed by comparison with that of its corresponding quiescent



**Fig. 1.** (a) Heating and (b) cooling profiles obtained through DSC for initial  $\kappa$ -carrageenan (0.5 wt%  $\kappa$ -carrageenan, 0.3 wt% KCl) fluid followed by quiescent gel. Step 1 shows melting of a previously prepared fluid gel followed by quiescent gelation (step 2). In-situ re-melting and gelling of the quiescent gel is then shown in steps 3 and 4 (Garrec & Norton, 2012).

gel. Results obtained from  $\kappa$ -carrageenan fluid gels (Fig. 1) have shown that the enthalpy of melting for the fluid gel ( $\Delta H_{m,FG} = 0.174 \pm 0.003$  J/g) is significantly lower than that of the quiescent gel ( $\Delta H_{m,QG} = 0.186 \pm 0.002$  J/g), indicating fewer number of helical domains in the fluid gel (Garrec & Norton, 2012).

As initially suggested by Norton et al. (1999), fewer helical domains and thus smaller helical aggregates are formed at the surface of the particles due to the disruption of the coil-helix transition by the applied shear field. Consequently, the density of the gelled network is lower towards the particle surface where the disruption to the aggregation process results in later ordering of polymer chain, leading to the formation of particles with 'hairs' (i.e. partially retained polymer chains). Unlike  $\kappa$ -carrageenan, fluid gels produced from agarose display similar melting enthalpies and temperature to those of their counterpart quiescent gels (Norton et al., 1999) indicating an equivalent internal structure. This disparity in the calorimetry results for systems produced from  $\kappa$ -carrageenan compared to those produced from agarose relate to differences in the rate of molecular ordering, being 2–3 orders of magnitude faster for agarose than  $\kappa$ -carrageenan (Norton, Goodall, Austen, Morris, & Rees, 1986). The fast ordering transition of agarose kinetically traps the structures into anisotropic shapes and particles typically reach larger dimensions. In contrast to this, fluid gel particles formed from  $\kappa$ -carrageenan under comparable processing conditions are small (1  $\mu$ m) and they achieve a thermodynamically favourable spherical shape. Thus, the structural features of the final particles (e.g. size, morphology, presence of 'hairs') are strongly dependent on the polysaccharide. These properties have a profound effect on the rheological response of fluid gels, particularly of diluted fluid gels. It is therefore essential to understand the influence of the bulk properties of the fluid gel particles on the degree of dilution, if we are to improve our ability to design colloidal structures that will be incorporated into foodstuffs.

The effects of particle shape were addressed in the work of Frith, Garijo, Foster, and Norton (2002) in which the volume fraction dependence of the storage modulus of agarose anisotropic fluid gels was compared with that of model microgel suspension of spherical agarose particles. Since both systems were produced from the same hydrocolloid, an equivalent degree of inter-particle interaction was assumed. Spherical particles were achieved by the emulsion route, cooling a water-in-oil emulsion where the gelling polymer is dissolved in the aqueous phase. Initially in this

method, a mixture of polymer aqueous solution and oil is sheared while heating at a temperature above the polymer gelation temperature. This produces droplets with a uniform polymer distribution and a well defined edge at the interface due to the high interfacial tension between the dispersed aqueous phase and the continuous oil phase. Thus, the gelled particles that form upon cooling are spherical particles with homogeneous polymer distribution. Consecutive processes of dilution with water and centrifugation are used to separate the particles from the continuous oil phase. Microgel model suspensions are then prepared by dispersing the produced spherical particles in water. As Fig. 2 shows, the response to dilution with water of the sheared anisotropic particles is markedly different to that of the uniform spherical particles. The highly irregular and extended morphology of fluid gel particles distribute through the space to a greater degree than the model spherical particles with an equivalent volume. This results in greater inter-particle interaction compared to spherical particles at low volume fractions which leads to the formation of percolated networks although the particles are not aggregated. As such, fluid gels display elastic dominated responses over a broad range of volume fractions (Firth et al., 2002).

In the work of Wolf, Frith, Singleton, Tassieri, and Norton (2001), the influence of particle shape on rheology and the process–morphology relationship was also investigated, where a wider range of microstructures was produced from the sheared gelation of mixtures of polysaccharides (Fig. 3). By manipulating the flow conditions during the gelation of one of the polymers, regular spheroidal or cylindrical shaped particles were formed. The use of a rheometer with concentric cylinder geometry allowed the production of uniform and regularly shaped gelled microstructures. Molten mixtures of gellan and  $\kappa$ -carrageenan were loaded to the rheometer at 60 °C and subjected to a constant stress of 0.05 Pa. Ellipsoidal shapes were achieved by cooling the system below the gelation temperature of the dispersed phase (gellan) whilst applying a constant stress (above 0.05 Pa). Alternatively, cylindrical particles were produced as a result of firstly cooling under small stress (0.05 Pa) and subsequently increasing the stress up to 0.5 Pa immediately before the gelation of the dispersed phase. Droplets are initially elongated into cylindrical threads which are then trapped via gelation and prior to being broken up. Kinetic trapping of the structure in controlled and defined shapes is achieved by controlling the time scale of the imposed increase in stress such that the process

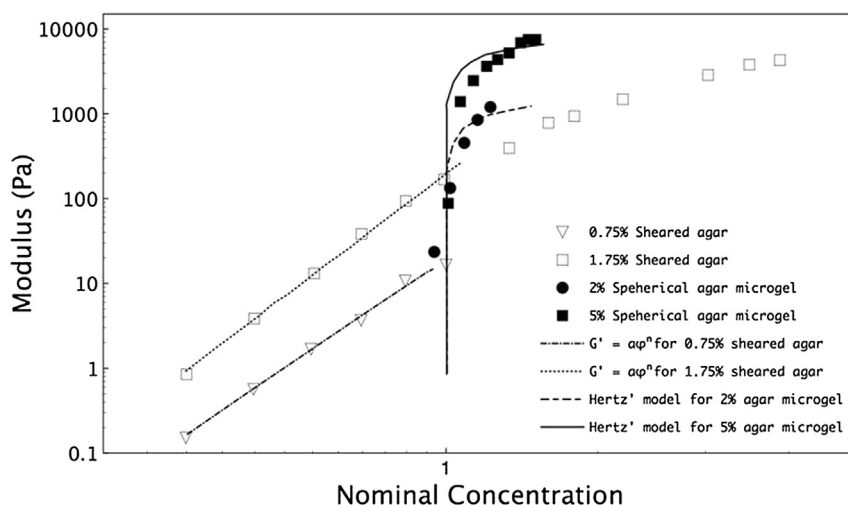
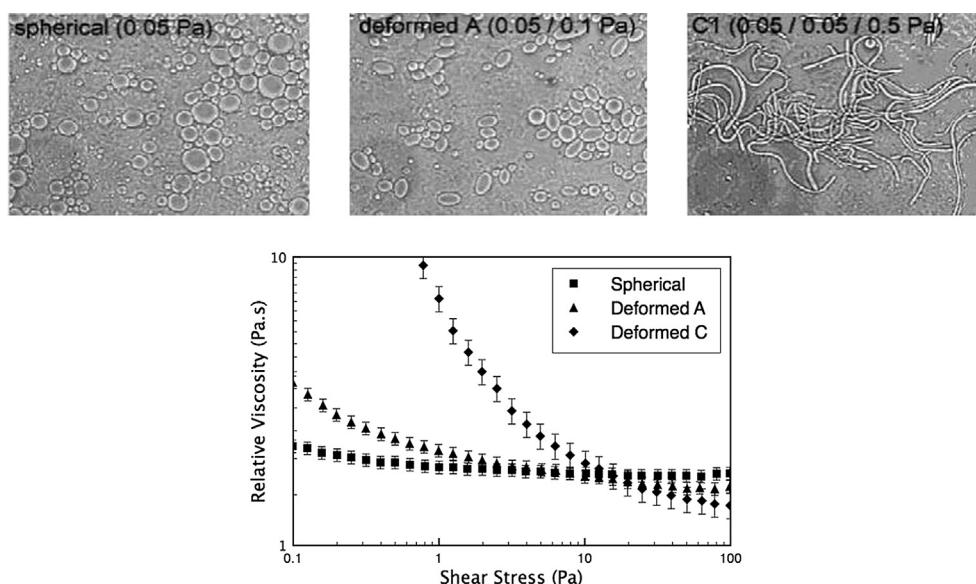


Fig. 2. Comparative plot for the concentration dependence of the plateau moduli for both sheared and model agar sphere suspensions. Predictions based on the Hertzian interactions between particles have also been illustrated, assuming a particle modulus of 33 KPa (Frith et al., 2002).



**Fig. 3.** Viscosity measurements for particulate asymmetric gel suspensions with varying degrees of aspect ratio. Micrograph inserts depict average particle morphologies for their respective viscometry profile (Wolf et al., 2001).

of droplet break-up occurs at a lower rate than gelation. If the gelation rate is too slow, spherical particles are formed due to the complete structural relaxation of the droplet phase.

The shear-induced structuring process in mixed systems requires less energy input than is necessary to produce fluid gels from a solution containing a single biopolymer (Norton, Frith, & Ablett, 2006). The reason for this lies in the fact that the two phases of mixed polymer systems are not pure such that each polymer exists in both gelled and aqueous state. Thus, only the weak interfacial tension has to be overcome by the applied flow field before inducing gelation. Wolf et al. (2001) showed a dramatic effect of particle shape on the relative viscosity, specifically at lower shear stresses where increasing the particle aspect ratio results in suspensions with increased viscosities (Fig. 3). At high shear stresses, a greater degree of particle orientation occurs upon increasing the particle aspect ratio, resulting in decreased viscosities. These results also showed that particle entanglements form over time after production due to the greater flexibility of cylindrical particles. Arising from this, particle orientation is suppressed. Moreover, once particle entanglement has occurred application of shear does not orient particles and a reduction in viscosity at higher shear rates is not observed.

It is clear that the evaluation of particle shape and the quantification of particulate volume fraction have led to a more detailed understanding of the relationship between particle morphology and fluid gel properties. In conclusion, both process and formulating parameters can be manipulated in the production of fluid gels from a single polysaccharide so as to create particles with different sizes and shapes. An even wider range of microstructures can be designed in order to achieve novel properties if the gelling polysaccharide is mixed with another polymer. The rheological properties of these particulate structures have potential applications in imparting texture and mouthfeel enhancement when used in emulsion based products. Undoubtedly, in order to establish whether these rheological behaviours translate into an expected in-mouth performance, an understanding of particle structure in relation to in-mouth sensory perception is required. Current models of the relationship between fluid gel lubrication and sensory response (functionality) involve the use of soft-tribology, recent advances in this area will be discussed later in this paper.

### 3. Recent developments: thermo-stable fluid gels

#### 3.1. Alginate fluid gels

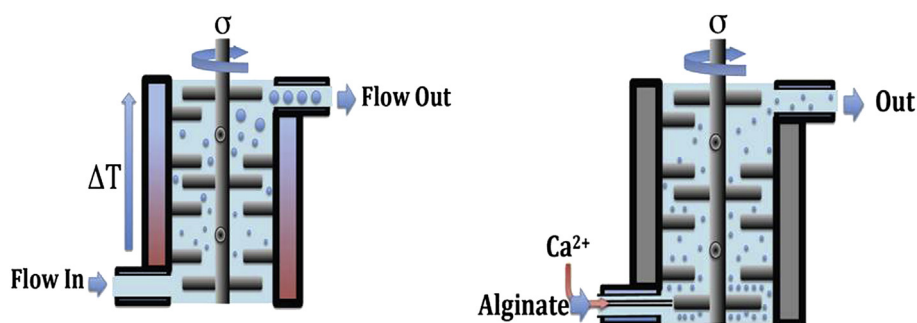
We have discussed the formation of fluid gels from polysaccharides undergoing thermal gelation (e.g. agar,  $\kappa$ -carrageenan and gellan) where the conformational ordering kinetics are controlled by the rate of cooling which can be matched to the time scale of the applied shear rate (Fig. 4A). In practice, the major difficulty in producing fluid gels from alginate arises from the rapid and temperature independent ordering transition of alginates. As such, controlling the introduction of calcium ions into the alginate solution becomes a key factor when fluid gels are to be produced via diffusion-controlled methods (i.e. using water soluble salts such as  $\text{CaCl}_2$ ).

A technique employing a pin-stirrer device has been developed to produce alginate fluid gels (Fernández Farrés, Douaire, & Norton, 2013). Alginate and  $\text{CaCl}_2$  solutions are separately pumped into the pin-stirrer with the aid of a peristaltic pump and a syringe pump, respectively. The injection point of the  $\text{CaCl}_2$  is located between the tip of the pin-stirrer and the outer wall so that the two streams merge in the highest energy dissipation zone (Fig. 4B). This allowed production of alginate particles with a diameter  $<10\ \mu\text{m}$ .

The authors suggested that particles form as a result of both mixing of calcium and network formation competing with the shear-induced break-up of the cluster being formed. At decreased polymer concentrations, lower shear stresses act on the initially formed particles due to the reduced viscosity which allows particles to grow to larger dimensions. Additionally, the increased diffusion rate of calcium through the less dense matrix increases the characteristic time for mixing the two liquids at molecular level (micromixing). As a result of these mechanisms, gel formation is favoured over the shear controlled breaking process, leading to an increased particle size (Fig. 5, Table 1).

This method bears the limitation of poor control on the competing parameters (mixing and gelation) so that the ability to monitor alginate concentration in a single microgel particle is still lacking. Because the shear field inside the stirring vessel is not uniform, the size of the particles is strongly affected by the exact location of the injection point.





**Fig. 4.** Schematic representations of thermally set fluid gels using a temperature gradient to induce gelation whilst applying shear (left) and alginate fluid gel particles produced using a syringe to inject Calcium ions directly into the high shear zone (right). The size of the injection syringe is not to scale.

Hence, a more refined technique using in-situ calcium release has recently been developed that enables production of alginate fluid gels with control over the gelation kinetics (Fernández Farrés & Norton, 2014). In this method, calcium ions are progressively liberated from an insoluble form of Calcium (e.g.  $\text{CaCO}_3$ ) by reaction with protons from the slow hydrolysis of a glucono- $\delta$ -lactone, as previously used for pectin and alginate gels (Draget, Østgaard, & Smidsrød, 1990; Ström & Williams, 2003). This gradual introduction of calcium ions to the alginate solution leads to uniform calcium distribution throughout the system which minimises the formation of inhomogeneous gelled networks and prevents large clusters.

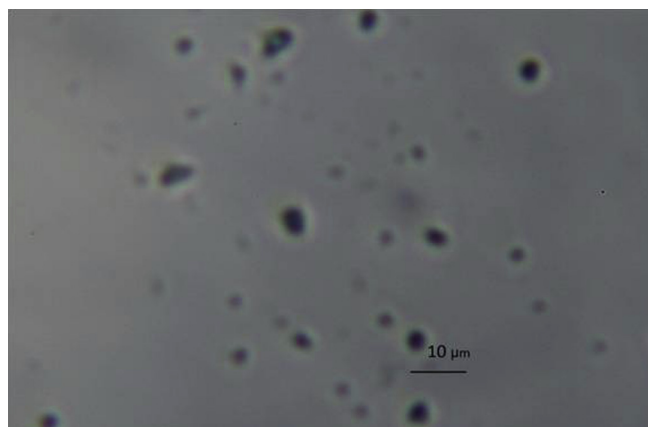
The shear is provided by the rheometer which allows accurate control of the applied shear rate while also monitoring the viscosity so that the kinetics of fluid gel formation can be studied. As shown in Fig. 6, systems produced with differing shear rates exhibit indistinguishable Newtonian viscosities before the onset of gelation. Under these conditions of limited calcium, monocomplexes form as a result of the binding of calcium to a single guluronate unit of the polymer chain which decreases the polymer charge density and gives rise to a local charge reversal (Fang et al. 2007; Siew, Williams, & Young, 2005). Monocomplexes are well separated from each other; hence remain unaffected by the level of shear. Gel nuclei are also expected to form during these early stages of the ordering process although their volume fraction is sufficient as to display any crowding effect thus no change in viscosity occurs. As more calcium ions are released, the pair wise association of monocomplexes leads to the formation of egg-box dimers. These egg-box dimers subsequently aggregate via inter-cluster association in a process that occurs simultaneously with the shear-induced

break-up of the network being formed, and fluid gel particles are produced. This simultaneous increase in number and volume fraction of the forming particles results in an increase in viscosity.

The time at which viscosity starts to increase (i.e. onset of gelation) increases upon increasing the level of the applied shear rate, indicating a reduction in the rate of the particle growth (Fig. 6). Therefore, the process of particle growth from the initially formed gel nuclei is restricted by the imposed flow field which confirms proposals by Norton et al. (1999) who suggested that the shear rate limits the likelihood of an association event occurring between the early formed particles. Further confirmation is obtained by measurements of  $G'$  when the shear rate is stopped prior to the completion of fluid gel particle formation. Results have shown increased rates of ordering post-production for systems formed at lower applied shear rates. At this point, intra-particle aggregations under shear change into inter-particle–particle aggregations, resulting in quiescent network formation via association of particles. Conversely, when the shear is removed after completion of fluid gel structure, the rate of ordering post-production due to inter-particle interaction is not affected by the magnitude of the applied shear rate during formation (Fernández Farrés & Norton, 2014).

Similarly to fluid gels made from agar or  $\kappa$ -carrageenan, alginate fluid gels display a flow behaviour that lies between that of a “weak” and a “strong” gel, with  $G'$  significantly greater than  $G''$  but more dependent on frequency than in the case of a quiescent gel. Current findings indicate that the stiffness and inter-particle interactions of alginate particle produced via in-situ calcium release can be controlled to give a range of viscoelastic responses.

Another feature that has been identified in alginates and which is commonly observed in thermo-reversible fluid gels is the presence of yield stress, arising from an inter-particle potential that persist post-production. True yield stress values can be determined by subjecting the fluid gels to a constant stress and recording the strain as a function of time. This test is then repeated increasing the stress in intervals. As shown in Fig. 7, the material behaves as a solid if the applied stress is below the yield stress and the measured strain is small and independent of time after an immediate elastic response. When the applied stress exceeds the yield stress, the

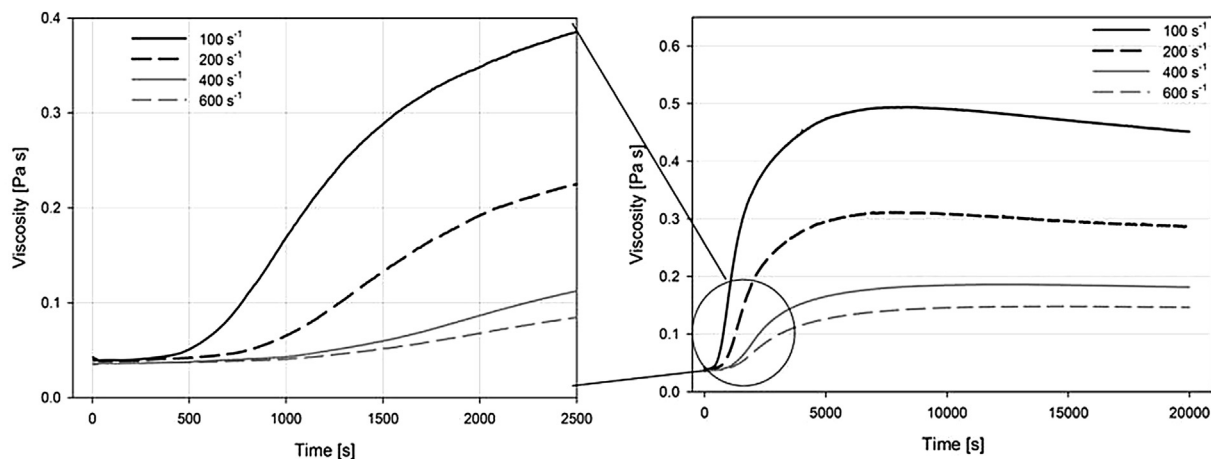


**Fig. 5.** Micrograph of diluted 3% (w/w) alginate fluid gel (Fernández Farrés et al., 2013).

**Table 1**

Particle size of alginate fluid gels produced by diffusion-controlled method (Fernández Farrés et al., 2013).

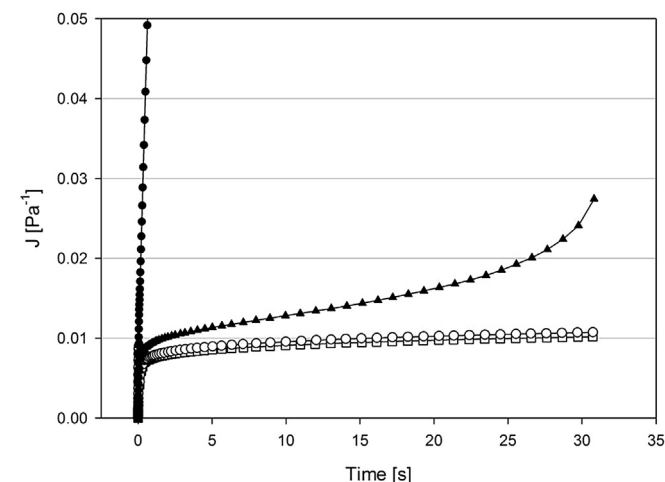
Polymer concentration [w/w %]	Particle diameter [μm]
1	$9.5 \pm 2$
2	$8.7 \pm 3$
3	$3.8 \pm 4$
4	$3.4 \pm 4$



**Fig. 6.** Viscosity during fluid gel production as a function of time. Systems were produced at a range of applied shear rates from 1.5% (w/v) alginate,  $\text{CaCO}_3$  22.5 mM, 45 mM GDL and isothermally at 25 °C (Fernández Farrés & Norton, 2014).

strain tends to infinity until constant strain rate is achieved corresponding to a flow condition in the material. The most likely cause for yield stress behaviour in fluid gels is the presence of hairs at the particle surface which can form bridges between adjacent particles (Norton et al., 1999) until the applied stress is great enough to either break or disentangle them. Increasing polymer concentration results in increases both particulate phase volume and particle stiffness which promotes inter-particle interaction such that an increased force is required to induced flow via particles sliding past each other (i.e. increase in yield stress). As a consequence, the rheological response of alginate fluid gels differs greatly from that of suspensions of hard spheres. The relatively soft and often 'hairy' nature of fluid gel particles allows them to overlap which prevents close packing behaviour.

Full fat products often contain proteins which confine oils droplets in network structures, which also exhibit yield stresses. From this perspective, alginate fluid gels could potentially be used as fat replacement in food product. Recent findings on the lubrication properties of alginate sheared gel particles are outlined in the following section.



**Fig. 7.** Constant stress (creep) test profile of alginate 2% (w/w). Applied shear stresses are 15 Pa (□), 17 Pa (●), 19 Pa (△), 21 Pa (■) and 23 Pa (○). True stress value (21 Pa) is identified as the critical stress causing a significant increase in the creep rate over a short period i.e. minimum stress required for the transition from limited deformation to appreciable flow conditions (Fernández Farrés et al., 2013).

Because of the thermally stable (semi-crystalline) calcium-mediated junction zones, alginate fluid gels do not melt when heated at temperatures commonly used in the food industry (e.g. 100 °C pasteurisation). These characteristics are clearly advantageous over other fluid gels produced from the commonly used  $\kappa$ -carrageenan, agar or gellan polysaccharides which must be kept and utilised at temperatures below their melting temperatures to preserve the structure.

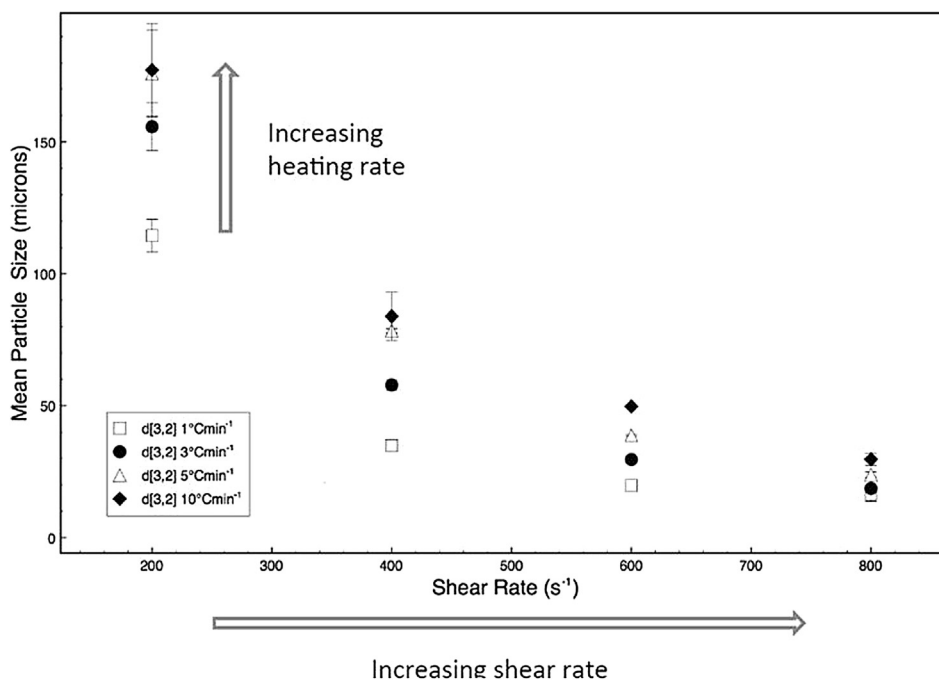
### 3.2. Protein fluid gels

Current work has begun to apply similar methods to those previously shown, to protein systems. This has led not only to thermo-stable fluid gels as a result of their irreversible denaturation on heating, but delivers the advantage of being nutritionally desirable (Moakes, Sullo, Spyropoulos, & Norton, in preparation).

Viscometry profiles during the formation of fluid gels from whey protein show similar trends to those observed for polysaccharides (Fig. 8) with the overriding difference describing structuring occurring on heating as opposed to cooling. Initially, a slight loss in the systems viscosity occurs due to the heating of the continuous phase, which is followed by a rapid increase in viscosity at a critical temperature ( $T_g$ ).

At  $T_g$ , the globular whey protein structure partially denatures whilst retaining its dimeric form. Nuclei form as consequence of the disruption to the secondary structure leading to the formation of oligomers through association of intermolecular  $\beta$ -sheets (Lefevre & Subirade, 2000). The nuclei grow through a succession of weak interactions and covalent bridging until all native protein within the system is consumed, at which point aggregate size becomes limited by shear. Lastly, post-ordering events occur with particle–particle interactions caused by branching as consequence of sulphide bridging. Further strengthening of the aggregate structures is also observed through increased internal structuring.

As shown for polysaccharide, the particle size of whey protein fluid gels decreases as a power function with increasing shear (Fig. 9). As expected, changing the thermal history of the whey protein systems leads to particle size increase (from low heating rates to higher), also best modelled using a power law. It has been reported that increasing the heating rate applied to the gelling system supports the initiation of an increased number of smaller nuclei (Bromley, Krebs, & Donald, 2006). However, larger aggregates are formed due to the increase of energy being applied to the system. The increase in energy within the system allows a shift



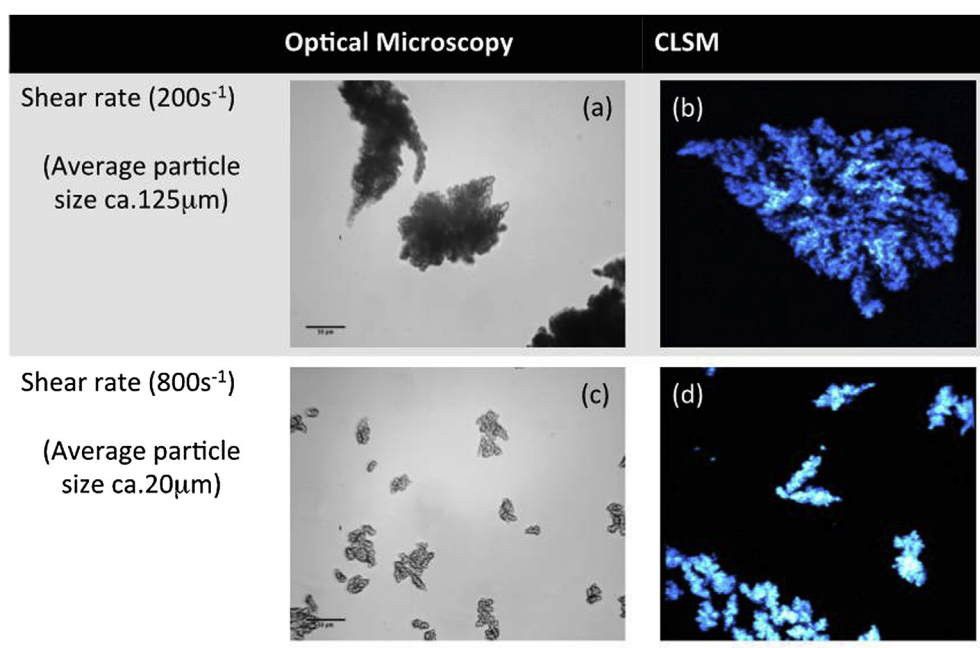
**Fig. 8.** Average whey protein fluid gel particle size shown as surface weighted means ( $d_{3,2}$ ). Means were determined using Static Light Scattering (SLS) at varying shear rates as a function of heating rate, ( $\square$ ) 1 °C min<sup>-1</sup>, ( $\bullet$ ) 3 °C min<sup>-1</sup>, ( $\Delta$ ) 5 °C min<sup>-1</sup> and ( $\blacklozenge$ ) 10 °C min<sup>-1</sup> (Moakes et al., in preparation).

from internal structuring to inter-particle bonding to occur. If sufficient energy is applied to overcome the activation energy needed for bond formation within a short time scale, particle size can shift to being predominantly limited by shear, but also become a function of heating. The kinetics of aggregation can thus be derived from the viscosity increase over time as a function of the gradient, which has been found to increase with increasing heating rate.

Fig. 10 shows both optical and confocal micrographs of whey protein fluid gel particles prepared at shear rate of 200 s<sup>-1</sup> and

800 s<sup>-1</sup>. The optical micrographs show aggregates with irregular morphology (Fig. 10A and C).

The use of three-dimensional stacking with confocal microscopy allowed higher resolution topographical detail of the particles to be obtained. It was shown that at the lower shear rate particles formed a porous network with voids protruding through the structure. This porous matrix appears to have been lost as the particles are subjected to higher levels of shear. It is believed that high shear prevents the ordering of clusters down to a single size, at which further



**Fig. 9.** Optical (a, c) and confocal light scanning micrographs (b, d) depicting whey protein fluid gel particles. Scale bar represents 50 μm (Moakes et al., in preparation).



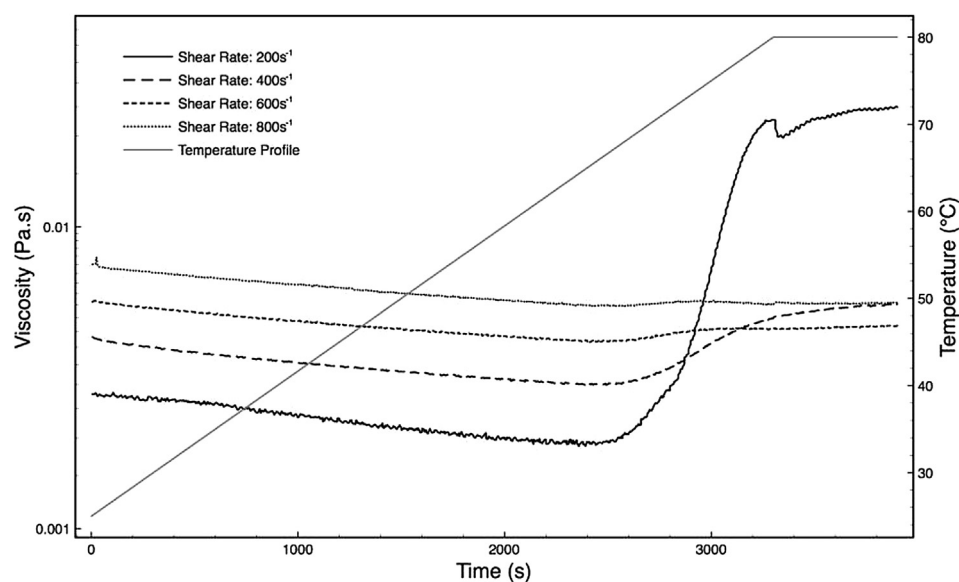


Fig. 10. Viscometry profiles for a 10 wt% whey protein solution undergoing gelation within a shear field (Moakes et al., in preparation).

increasing shear has little effect (Fig. 9). This cluster (Fig. 10D) then forms the building block for larger aggregates, with porosity being a function of packing efficiency. Both optical and confocal micrographs show the presence of inter-particle interactions with the weak grouping of particles within localised regions.

Flow and deformational properties of the fluid gels, as previously stated, are closely linked to the microstructure, shape and interactions between particles. The use of image analysis over a large sample (>1000 particles) has shown the average aspect ratio of the structures to be 1.63 describing an elongated structure. It was found that the change in aspect ratio with increasing shear was minimal, determining that changes in rheology are more closely linked to particle microstructure and interactions than morphology. Increasing shear within the system has shown to decrease the relative viscosity change of the fluid gel following the prevention of bridging between particles (Fig. 8). It is also thought that the “strength” of the material is formed through a combination of both volume fraction and particle elasticity, as shown for alginate fluid gels. Therefore, controlling the resulting microstructure through processing conditions and also volume fraction gives rise to possible control over desired rheological properties. This could lead to the application of protein fluid gels within high-level protein food supplements with thermo-stable behaviour.

#### 4. Fluid gel lubrication

A significant challenge for microstructural engineering of foods is the development of low fat formulations with textural and sensory attributes similar to those of the full fat version. As mentioned earlier, the physical properties and thus textural attributes of fluid gel particles can be manipulated so as to mimic those of the oil droplets that are replaced. Norton, Cox, and Spyropoulos (2008) showed that the rheological properties of a mayonnaise in which most of the oil droplets were replaced by sheared gel particles matched those of a commercial full fat product.

As can be seen from Fig. 11, the sheared gel mayonnaise exhibits both a yield stress and an overall rheological response comparable to that of the full fat version. Yield stress is measured as the finite shear stress at which deformation starts when subjected to a transient linear stress ramp. The low fat mayonnaise used in this

study was produced from sheared agar and 3% (phase volume) oil was added to impart both flavour and mouthfeel. This is a clear indication that sheared gel particles can replace a significant proportion of the fat droplets in an oil-in-water emulsion with minimal changes to the material properties. In addition to their rheological behaviour, an understanding of the structural changes which occur during oral processing of these food soft materials and their influence on sensory perception is necessary if we are to successfully design structured foods with reduced energy content and are also enjoyable to eat.

Oil droplets play a determining role in the perception of creaminess (de Wijk & Prinz, 2005) which is also related to thickness (viscosity), smoothness (size, shape and hardness of particles) and slipperiness (Kokini, 1987; Shama & Sherman, 1973; Tyle, 1993). Tribology (thin film rheology) has emerged in recent years as a powerful technique to study the structure–functionality relationship, allowing major advances in understanding how colloidal systems (including fluid gels) affect lubrication and their influence on in-mouth sensory perception. Malone et al. (2003) found a strong correlation between the sensory perception of slipperiness and the friction of an oil-in-

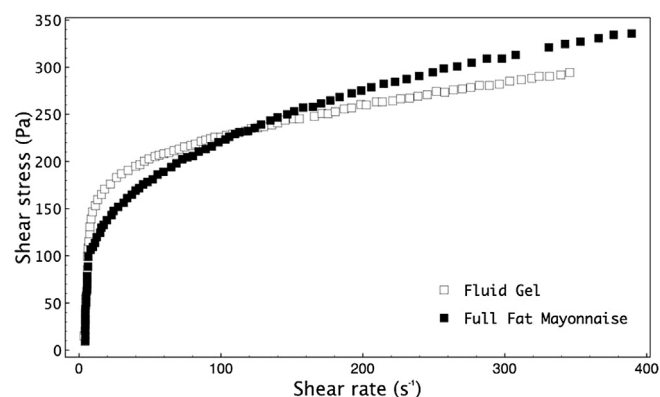


Fig. 11. Comparative shear stress/shear rate curve of agar fluid gel with a 3% (phase volume) of oil and a full fat commercial (Hellman's) mayonnaise (Cox, Spyropoulos, & Norton, 2009).

water emulsion measured in a soft-tribological contact using silicon rubber to mimic the soft surfaces of the mouth. From this correlation, it was determined that speeds between  $10 \text{ s}^{-1}$  and  $100 \text{ s}^{-1}$  are involved in the movements between the tongue and the palate during oral processing of food.

A tribometer consist of a rotating ball loaded against a rotating disc at a  $45^\circ$  angle creating a sliding/rolling contact and the resulting frictional force at the ball-in-disc contact is measured by a force transducer. On introduction of a lubricant fluid between the surfaces, the fluid pressure increases as the lubricant is gradually drawn into the centre of the contact which partly separates the ball from the disc. As the rotation speed is raised, the increase in volume of lubricant in the contact results in a further surface separation thereby further reducing friction. Stribeck curves are generated by plotting the friction coefficient ( $\mu$ ) over a range of speeds ( $U$ ), where three different regimes are identified: During boundary lubrication which occurs at the lowest speeds, where the lubricant is excluded from the contact, the load is supported by the asperity contact and  $\mu$  is independent on  $U$ . In the mixed regime,  $\mu$  decreases with increasing  $U$  as the increase in fluid pressure separates the surfaces resulting in both asperity contact and lubricant film supporting the load. At higher speeds, friction increases with  $U$  as the fluid volume which is being sheared increases (hydrodynamic regime) although this is not always observed in fluid gel systems.

Gabriele, Spyropoulos, and Norton (2010) studied the lubrication response of fluid gels produced from agarose with a  $100 \mu\text{m}$  particle size. The obtained Stribeck curves showed an increase in friction coefficient ( $\mu$ ) at low speeds during the mixed regime before the film thickness was great enough so as to induce bulk entrainment. At these low speeds, the lubrication film thickness is lower than the size of the large agarose particles which, as a result, are selectively excluded from the ball-in-disc. The frictional increase was argued to be the result of the lubrication regime provided by the entrainment of a thin monolayer of particles. The proposed mechanism is based on a micro-elastohydrodynamic (micro-ELH) lubrication regime in which the fluid dynamics of particles in the thin film strongly depends on the particle's Young modulus.

An increase in friction at low speeds has also been observed for alginate fluid gels containing particles with a mean average diameters ranging from  $3.4 \pm 4 \mu\text{m}$  to  $9.5 \pm 2 \mu\text{m}$ . As shown in Fig. 12, this peak in friction is less pronounced for the smaller and stiffer alginate particles produced from higher polymer concentrations which also exhibit an overall reduced friction. It is expected that less deformable particles provide lower frictional data as a result of a reduced surface contact due to the stiffer particles greater resistance to compressive forces. The root mean square roughness of the silicone disc surface is approximately  $0.8 \mu\text{m}$ , thus sufficiently small particles ( $<1 \mu\text{m}$ ) can fit into the disc surface irregularities thereby entering the contact zone via surface roughness. As a result, particle exclusion decreases and systems exhibit a less pronounced peak in friction. This effect was further shown in the work of Garrec and Norton (2013) where  $\kappa$ -carrageenan fluid gel particles having a diameter equivalent to the surface roughness dimensions ( $1 \mu\text{m}$ ) provided lubrication at all speeds through particle entrainment, resulting in Stribeck curves without any frictional increase. Because the friction in the boundary lubrication regime was reduced below that of the continuous phase (water) upon increasing the number and stiffness of the particles, particle entrainment was also suggested to occur in boundary conditions.

As illustrated in Fig. 13, the boundary friction of  $\kappa$ -carrageenan fluid gels decreases with particle volume fraction ( $\phi$ ) until a plateau region is reached ( $0.3 < \phi < 0.6$ ). This is followed by a further reduction in friction with increasing  $\phi$  ( $\phi > 0.7$ ). The initial

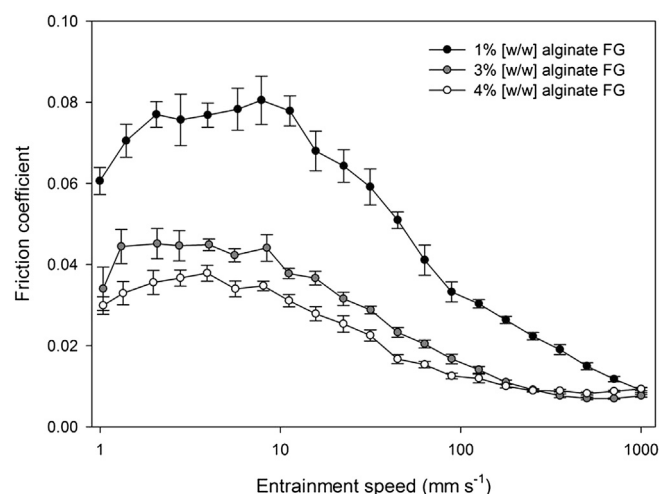


Fig. 12. Effect of polymer concentration and particle size on lubrication properties of alginate fluid gels. Stribeck curves (ramp up) are presented for applied normal load  $W$  of 5 N. The mean frictional increase of the 1% (w/w) alginate system is roughly twice as large in magnitude compared to that of the 3% (w/w) and 4% (w/w) alginate fluid gels (Fernández Farrés et al., 2013).

decrease in  $\mu$  is the result of an increased number of particles in the contact zone. The fact that a plateau region is observed suggests that the dependence of the boundary friction on volume fraction relates to the number of particles in the contact, rather than the bulk rheology and inter-particle interaction. Finally, at high volume fractions, a multi-layer of closely packed particles is entrained to the contact zone and the friction is further reduced. Thus, unlike the fluid gel rheology which greatly depend on the level of inter-particle interactions and hence volume fraction, fluid gel tribology is mainly dominated by the elasticity of the particles.

Data obtained from sensory trials revealed that  $\kappa$ -carrageenan fluid gel particles were not perceived as particles, rather, fluid gels were perceived as 'smooth' (Garrec, 2013). There is therefore an indication that stiffness of fluid gel particles can be manipulated to provide enhanced lubrication while not being detected during consumption.

By understanding the microstructure–property relationship the influence of fluid gel lubrication on perceived sensory attributes can be further studied. Tribology as a scientific tool to relate the structural properties to the sensory perception is just starting to

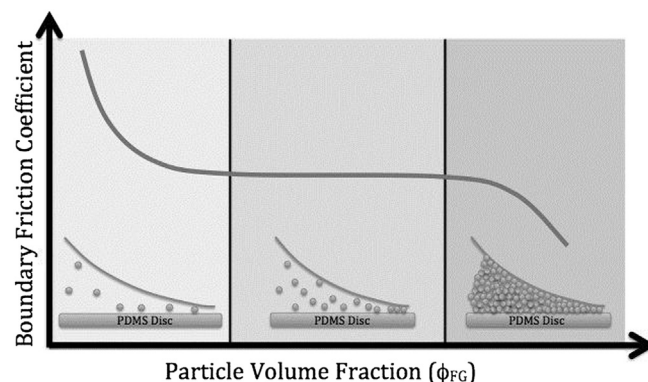


Fig. 13. Schematic representation of the particle entrainment at three distinctive regions of boundary friction coefficient with varying  $\phi$ . Initially  $\mu$  decreases with  $\phi$  as the number of particles in the contact increases. Then, a plateau region occurs where changes in  $\phi$  only influence the bulk and not the contact zone. Finally, at high volume fractions, multi-layer particle entrainment occurs (Garrec & Norton, 2013).

develop. Further success will require a combined knowledge of material science and sensory science, along with an understanding of how the processes involved during oral processing (e.g. temperature, mixing with saliva, squeezing and shearing between palate-tongue) affect the structure.

## 5. New concepts for designing colloidal structures

The production of colloidal gelled structures offers a broad range of material properties that can be modified to meet a range of product needs. As discussed, controlled structuring processes in which conditions are well defined are used to deliver fluid gel structures with desirable material properties. Fluid gels show promising characteristics for fat replacement as their textural and tribological responses can be controlled to mimic those of oil droplets. The removal of fat in foodstuff not only affects the material properties, but also is detrimental to flavour perception.

In order to control and enhance flavour and mouthfeel, novel methods to produce more complex structures are under development. One microstructural approach involves the entrapment of oil droplets within the fluid gel particles, forming a system resembling an oil-in-water emulsion. This would allow fat soluble flavour molecules to be added to the dispersed oil phase so that they could be released as the gel breakdowns in the mouth thereby imparting both desirable mouthfeel and flavour attributes. In addition to flavour, micronutrients could be dissolved in the oil droplets, thus offering the potential for low-energy and nutritionally rich foodstuffs with acceptable organoleptic properties.

The formulation and production process of such structurally complex systems sets the challenge of maintaining the stability of the oil droplets inside the gelled particles. Lipophilic emulsifiers may be required although it is not yet clear how an interface alter the physical properties. An alternative method for stabilising the oil is the creation of a solid fat interface by adsorption of triacylglyceride particles.

The correct microstructural engineering will be required to ensure that the rupture of the gelled emulsion structure occurs either in the mouth for flavour release or inside the gastrointestinal tract for the release of vitamins or other nutrients.

Another exciting microstructural approach which may allow encapsulation and controlled release of nutrients inside the gastrointestinal tract is building layered fluid gel structures arising from the association between alginate and chitosan. In acidic conditions, alginate gels contract due to reduced solubility of non-ionised carboxyl groups while, in alkaline conditions, the increased hydrophobicity of the ionised carboxyl groups leads to swelling and dissolution. On the contrary, chitosan dissolves in acidic medium due to the hydrophobicity the protonated amino groups and is insoluble in alkaline conditions. By mixing the two polymers, the electrostatic interaction between the carboxyl groups of alginate and the amino groups of chitosan form a polyelectrolyte complex which is insoluble at pH below 3.5 and dissolves at pH above 6 due to a reduction in charge density of the chitosan (George & Abraham, 2006; Huguet, Neufeld, & Dellacherie, 1996; Tønnesen & Karlsen, 2002). Thus, chitosan coated alginate fluid gel particles should be stable during their passage through the stomach ( $1.5 < \text{pH} < 3.5$ ) and subsequently disintegrate in the intestine ( $\text{pH} \approx 7$ ) thereby releasing the encapsulated nutrient. In practical terms, the main challenge will be the formation of a defined chitosan layer which will reduce the porosity of the alginate network hence preventing leakage of encapsulated nutrients.

Alternative mixed biopolymer composites can also be exploited to produce particles with enhanced stiffness as a result of associative interactions between the polymers (e.g. carrageenan and

locus bean, gelatin and low-methoxy pectin). As a result, novel textural properties could be achieved which may potentially provide enhanced lubrication typically associated with fat content.

Elucidating the function of the interface during the structuring process will be an exciting challenge which will enable the development of predictive models for the microstructure–property relationship of mixed fluid gels.

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